(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 318 142 A1

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 158(3) EPC

(43) Date of publication:
11.06.2003 Bulletin 2003/24

(21) Application number: **01963437.7**

(22) Date of filing: **05.09.2001**

(51) Int Cl.[7]: **C07C 271/20**, C07C 269/06, C07K 14/00, B01J 39/04, B01J 39/06

(86) International application number:
PCT/JP01/07696

(87) International publication number:
WO 02/020470 (14.03.2002 Gazette 2002/08)

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

(30) Priority: **05.09.2000 JP 2000268638**

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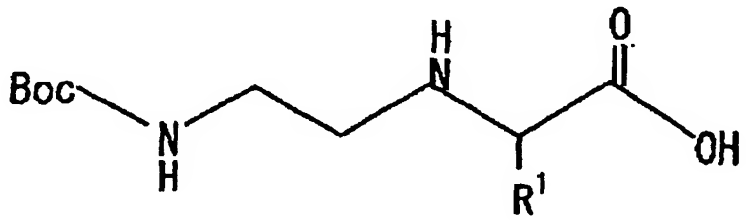
(54) T-BUTOXYCARBONYLAMINOETHYLAMINE FOR THE SYNTHESIS OF PNA MONOMER UNITS, AMINO ACID DERIVATIVES, INTERMEDIATES THEREOF, AND PROCESSES FOR PRODUCTIONS OF THEM

(57) A process for amino acid derivatives shown by the below general formula (I):



(I)

(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms.) having an object to provide a process of the amino acid derivatives of the formula I and their synthetic intermediate, t-butoxycarbonylamino-ethylamine, whereby it requires no tedious procedure and is also good in yield, and its application to a mass production is easy, and to provide novel amino acid derivatives of the formula IV, their synthetic intermediates, and a process thereof, characterized in that it comprises a step to obtain the amino acid derivatives shown by the general formula (I) by hydrolysis of compounds shown by the below formula (11):

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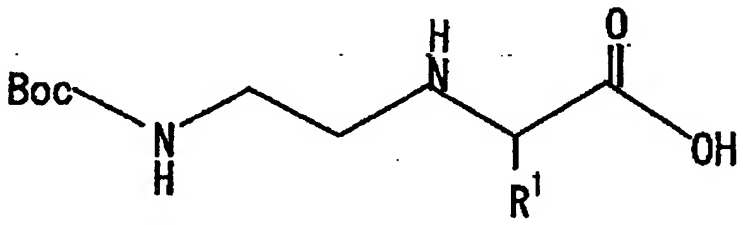
Description

Technical Field of the Invention

[0001] The invention relates to a process for amino acid derivatives and t-butoxycarbonylaminoethylamine which is their intermediate, and more particularly relates to a process for amino acid derivatives and t-butoxycarbonylaminoethylamine which is their intermediate, expediently used as a base or a base substance for introducing a functional molecule in case of synthesizing a monomer unit for syntheses of Boc-type PNA, an amino acid derivative introducing a Boc-type functional molecule and the like.

Background Art

[0002] Amino acid derivatives shown by the below formula (I)



(I)

(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl groups with 1-5 carbon atoms. Hereinafter it is same.) have a wide variety of use as a base or a base substance for introducing a functional molecule in case of synthesizing a monomer unit for synthesis of Boc-type PNA, an amino acid derivative introducing a Boc-type functional molecule and the like.

[0003] In particular, as shown in Fig. 1, PNA (Peptide Nucleic Acid) has the structure in which the sugar phosphoric acid skeleton in a natural nucleic acid such as DNA is converted into the N-(2-aminoethyl)glycine skeleton, is high in a double strand formation performance and a base sequence recognition performance compared with a natural nucleic acid, further is intact for an *in vivo* nuclease and protease, and therefore its application to a gene therapy as an antisence molecule is examined, attracting attention in recent years. The above characteristics of PNA is due to the fact that the sugar phosphoric acid skeleton in a natural nucleic acid has negative charge in neutral conditions whereby an electrostatic repulsion between complementary chains is produced, and in contrast in PNA having N-(2-aminoethyl)glycine skeleton without charge no electrostatic repulsion is produced between complementary chains.

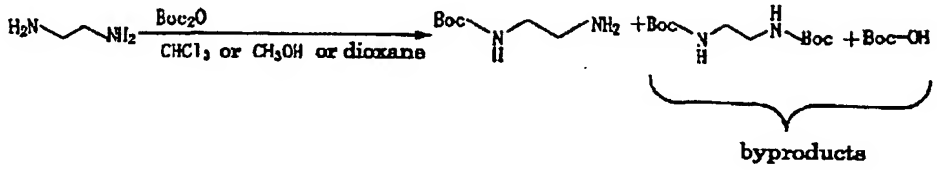
[0004] Synthesis of PNA is carried out by sequentially combining an amino acid (especially glycine) derivative (monomer unit) which is introduced by any one of four kinds of bases (A, T(U), C and G) constituting DNA or RNA according to an aimed base sequence using a conventional solid peptide synthesis method. As for monomer units for the synthesis of PNA, there are two types, Fmoc type and Boc type, as shown in Fig. 2 (B represents base), though synthetic methods of monomer units are established and use of a Fmoc type which, enables to utilize a general DNA automatic synthesis machine to synthesize a PNA oligomer currently has become a major trend. However, because in case of synthesizing PNA by use of a Boc type monomer unit there is an advantage that a functional molecule unstable in basic conditions can be introduced in PNA, establishment of a PNA synthesis method using a Boc type monomer unit becomes an urgent matter.

[0005] As one of obstacles to hinder establishment of a PNA synthesis method using a Boc type monomer unit, it can be cited that a simple and cheap synthesis method for a monomer unit before introduction of a base, that is, a Boc type amino acid derivative shown in the formula I, is not to be established. Also, because an amino acid derivative of the formula I has use as a base substance for introducing other functional molecule in stead of the base, synthesis of an amino acid derivative introducing a functional molecule becomes easy if its simple and cheap synthesis method is established.

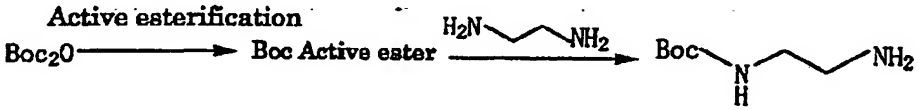
[0006] The synthetic method for an amino acid derivative of the formula I usually makes ethylenediamine a starting material and contains a step to introduce t-butoxycarbonyl group (Boc) to one nitrogen atom and a step to introduce $-CHR^1-COOH$ to the other nitrogen.

[0007] As a method to obtain t-butoxycarbonylaminoethylamine by introduction of Boc to one nitrogen atom of ethylenediamine, for example, (1) methods are reported in which t-butoxycarboxylic acid anhydride is directly reacted to ethylenediamine in a reaction solvent such as chloroform, methanol or dioxane (J. Med. Chem., 38(22), 4433-8; 1995,

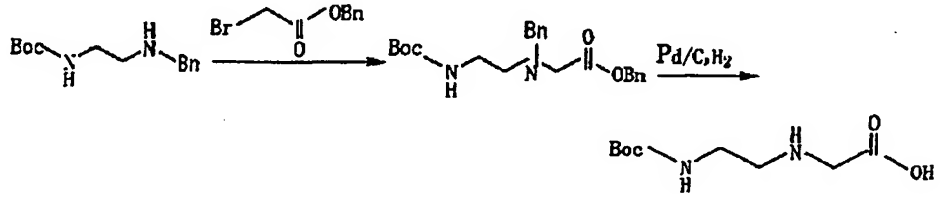
Bull. Korean Chem. Soc., 15(12), 1025-7; 1994, Eur. J. Med. Chem., 26(9), 915-20; 1991, Synth. Commun., 20(16), 2559-64; 1990, Aust. J. Chem., 39(3), 447-55; 1986),



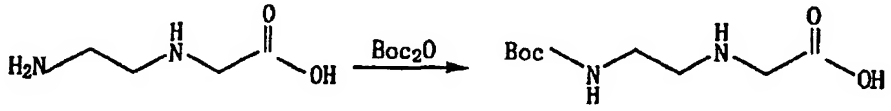
and (2) a method in which t-butoxycarboxylic acid anhydride is converted to an active ester followed by reaction with ethylenediamine (JP, A 11-012234,).



[0008] Also, as a method to obtain an amino acid derivative of the formula I by introduction of -CHR^1-COOH to t-butoxycarbonylaminoethylamine, a method is reported in which benzyl group is introduced to the unprotected nitrogen atom of t-butoxycarbonylaminoethylamine, (3) followed by reaction with benzyl bromoacetate and then by the catalytic reduction (J. Org. Chem., 62(2), 411-416; 1997).



[0009] Further, as a method to obtain an amino acid derivative of the formula I by introduction of Boc to the other nitrogen atom of the ethylenediamine derivative in which -CHR^1-COOH is introduced to one nitrogen atom, (4) a method is reported in which t-butoxycarboxylic acid anhydride is reacted to N-(2-aminoethyl)glycine (Heimer, E. P.; Gallo-Torres, H. E.; Felix, A. M.; Ahmad, M.; Lambros, T. J.; Scheidl, F.; Meienhofer, J.Int. J. Pept. Protein Res. 23(2), 203-211, 1984).



[0010] However, as a method to prepare t-butoxycarbonylamino-ethylamine, in the method 1 the aimed substance can be obtained in relatively good yield, though di(t-butoxycarbonylamino)ethylene and t-butoxycarboxylic acid are produced as byproducts, existing in a reaction solvent such as chloroform, methanol or dioxane. Owing to this, a partition extraction procedure or partition chromatography are necessary, making it difficult to prepare t-butoxycarbonylaminoethylamine efficiently in a large amount and cheaply.

[0011] Also, although the method 2 has an advantage that di(t-butoxycarbonylamino)ethylene is not produced as a byproduct, the total yield is as low as about 60% due to a multistep reaction, and because used reagents must be removed by partition chromatography, it is difficult to prepare t-butoxycarbonylaminoethylamine efficiently in a large amount and cheaply just like the method 1.

[0012] Therefore, both methods 1 and 2 are inappropriate as a method to industrially prepare t-butoxycarbonylaminoethylamine.

[0013] Also, as a method to obtain an amino acid derivative of the formula I from t-butoxycarbonylaminoethylamine, the method 3 is a multistep reaction, and a partition extraction procedure is necessary, being inappropriate for an industrial preparation.

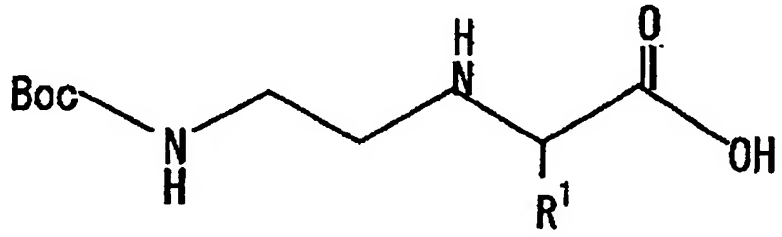
[0014] Further, as a method to obtain an amino acid derivative of the formula I, the method 4 has an advantage that partition chromatography is unnecessary, though the yield is around 60%, being inappropriate for an industrial preparation. That is, an efficient method to obtain a photo-functional PNA molecule has not been established due to a low efficiency in the synthesis of an amino acid derivative of the formula I. Therefore, needed are a method to obtain an amino acid derivative of the formula I and the development of the amino acid derivative to make a more efficient synthesis of the photo-functional PNA molecule possible in case of using an amino acid derivative of the formula I.

Disclosure of the Invention

[0015] The invention is accomplished in view of these circumstances, and the object is to provide a process of an amino acid derivative of the formula I and its synthetic intermediate, t-butoxycarbonylaminoethylamine, wherein it requires no tedious procedure and is also good in yield and its application to a mass production is easy, and is to provide novel amino acid derivatives shown by the formula IV, their synthetic intermediates and a process thereof.

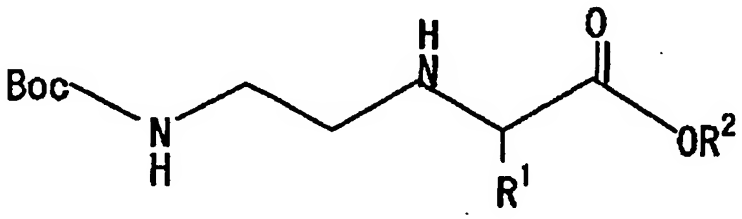
[0016] The inventors made extensive researches to solve the above problems, found out the solution, and thus accomplished the invention.

[0017] Accordingly, the invention relates to a process of amino acid derivatives shown by the formula I below:



(I)

(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms.), comprising a step to obtain amino acid derivatives shown by the general formula (I) by hydrolysis of compounds shown by the formula (II) below:



(II)

(wherein R^1 has the same meaning as described above, and R^2 means a straight chain or branched chain alkyl group with 1-4 carbon atoms.).

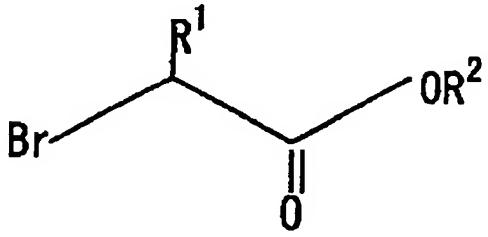
[0018] Also, the invention relates to the above process, characterized in that the hydrolysis of compounds shown by the below formula (II) is carried out by an aqueous alkaline metal hydroxide solution.

[0019] Further, the invention relates to the above process, characterized in that it contains further a step in which the alkaline metal ion is removed by cation-exchange chromatography using pyridinium ion as a counter ion.

[0020] Furthermore, the invention relates to the above process, characterized in that the alkaline metal is lithium, sodium or potassium.

[0021] Also, the invention relates to the above process, characterized in that the compound shown by the formula

(II) is obtained by reaction of t-butoxycarbonylaminoethylamine and a compound shown by the formula (III) below



(III)

(wherein R^1 and R^2 have the same meanings as described above.).

[0022] And the invention relates to the above process, characterized in that t-butoxycarbonylaminoethylamine is obtained by reaction of ethylenediamine and t-butoxycarboxylic acid anhydride.

[0023] Furthermore, the invention relates to the above process, characterized in that the reaction of ethylenediamine and t-butoxycarboxylic acid anhydride is carried out in tetrahydrofuran.

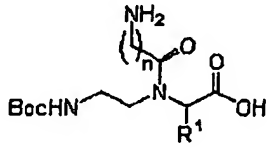
[0024] Also, the invention relates to the above process, characterized in that in the compounds shown by the general formulas (I), (II) and (III) R^1 is a hydrogen atom and R^2 is an ethyl group.

[0025] Further, the invention relates to the above process, characterized in that the amino acid derivatives shown the formula (I) are base substances for introducing a base to synthesize a Boc type PNA monomer unit.

[0026] Also, the invention relates to use of the above process in the preparation of a Boc type PNA monomer unit.

[0027] Further, the invention relates to a process of t-butoxycarbonylaminoethylamine, characterized in that ethylenediamine and t-butoxycarboxylic acid anhydride are reacted in tetrahydrofuran.

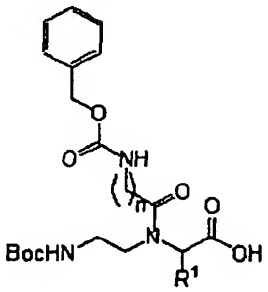
[0028] Also, the invention relates to amino acid derivatives shown by the general formula (IV):



(IV)

(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, and n means any one of integers 1-11.).

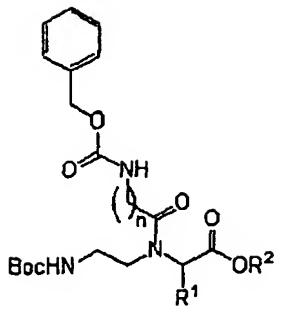
[0029] Further, the invention relates to intermediates of the amino acid derivatives shown by the above general formula (IV) which are shown by the general formula (V):



(V)

(wherein R^1 and n have the same meanings as described above.).

[0030] Also, the invention relates to intermediates of the amino acid derivatives shown by the above general formula (IV) which are shown by the general formula (VI):



(VI)

(wherein R^1 and n have the same meanings as described above, and R^2 means a straight chain or branched chain alkyl groups with 1-4 carbon atoms.).

[0031] Further, the invention relates to a process for the amino acid derivatives shown by the above general formula (IV), comprising a step to obtain the compounds of the general formula (V).

[0032] Also, the invention relates to the above process, characterized in that the reduction of the compounds shown by the general formula (V) is carried out in a methanol solution containing palladium carbon as a catalyst.

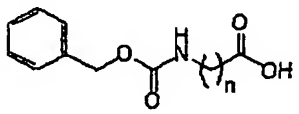
[0033] Further, the invention relates to the above process, characterized in that it contains a step to obtain the compounds shown by the general formula (V) by hydrolysis of the compounds shown by the general formula (VI).

[0034] Also, the invention relates to the above process, characterized in that the hydrolysis of the compounds shown by the general formula (VI) is carried out by an aqueous alkaline metal hydroxide solution.

[0035] Furthermore, the invention relates to the above process, characterized in that it contains a further step in which the alkaline metal ion is removed by cation-exchange chromatography using pyridinium ion as a counter ion.

[0036] Also, the invention relates to the above process, characterized in that the alkaline metal is lithium, sodium or potassium.

[0037] And the invention relates to the above process, characterized in that the compounds shown by the general formula (VI) are obtained by reaction of benzyloxycarbonyl- -amino acids shown by the general formula (VII) below:



(VII)

(wherein n has the same meaning as described above.).

and the compounds shown by the general formula (II).

[0038] And the invention relates to the above process, characterized in that in the compounds shown by any one of the general formulas (II) and (IV)-(VII) R^1 is a hydrogen atom, R^2 is an ethyl group, and n is 1.

[0039] Furthermore, the invention relates to the above process, characterized in that the amino acid derivatives shown the formula (IV) are base substances for introducing a base to synthesize a Boc type PNA monomer unit.

[0040] Further, the invention relates to the above process, characterized in that the compounds shown by the general formula (II) are obtained by the reaction of t-butoxycarbonylamino-ethylamine prepared from ethylenediamine and a compound shown by the general formula (III).

[0041] Also, the invention relates to a process for the compounds shown by the general formula (V), characterized in that it contains a step to obtain the compounds shown by the general formula (V) by hydrolysis of the compounds shown by the general formula (VI).

[0042] Further, the invention relates to a process for the compounds shown by the general formula (VI), characterized in that it contains a step to obtain the compounds shown by the general formula (VI) by reaction of a benzyloxycarbonyl- -amino acid shown by the general formula (VII) and a compound of the general formula (II).

[0043] Furthermore, the invention relates to the use of the compounds shown by the above general formula (IV) in the preparation of a Boc type PNA monomer unit.

[0044] Also, the invention relates to the use of the compounds shown by the above general formula (V) in the preparation of a Boc type PNA monomer unit.

[0045] And the invention relates to the use of the compounds shown by the above general formula (VI) in the preparation of a Boc type PNA monomer unit.

[0046] Since in the compounds shown by the general formula (IV) a linker binds beforehand, they are rich in versatility,

and an aimed PNA monomer unit can be obtained in one step by reaction of an active ester with said compounds. Therefore, since according to the compounds shown by the formula (IV) a photo-functional molecule can be converted into a PNA monomer unit by less synthetic steps compared with current methods, said compounds are particularly effective in case of targeting a relatively expensive photo-functional molecule.

[0047] In the meantime, making a photo-functional molecule which is a sulfonic acid chloride type and has a big steric hindrance and the like into a PNA monomer can be carried out using the compounds shown by the general formula (I). Therefore, various kinds of functional PNA monomers can be synthesized according to the invention.

Brief Description of Drawings

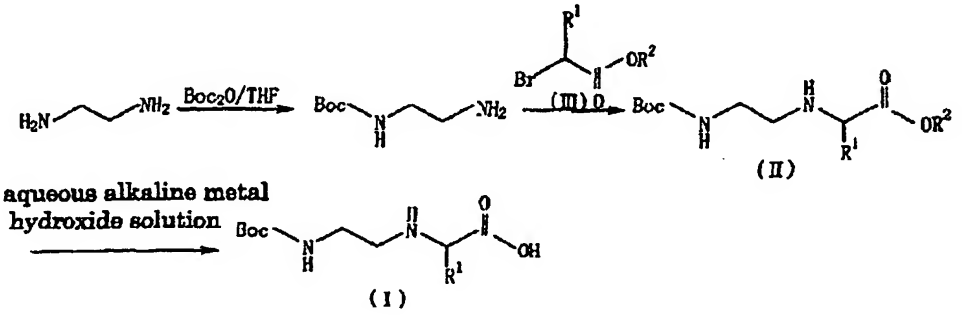
[0048] Fig. 1-1 and Fig. 1-2 are illustrations to show the structures comparing a natural nucleic acid with a peptide nucleic acid. Fig. 2 is illustrations to show comparing with a Fmoc type monomer unit and a Boc type monomer unit.

[0049] In each figure, 1 and 2 show a sugar phosphoric acid skeleton and a N-(2-aminoethyl)glycine skeleton, respectively.

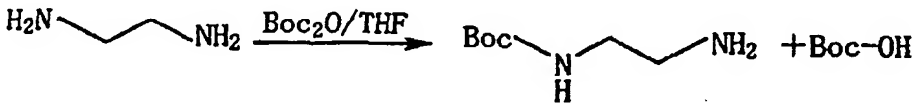
Embodiment of the Invention

[0050] In the following is explained an embodiment of the invention in more detail.

[0051] In the invention the amino acid derivatives shown by the formula (I) are prepared by the steps shown below.



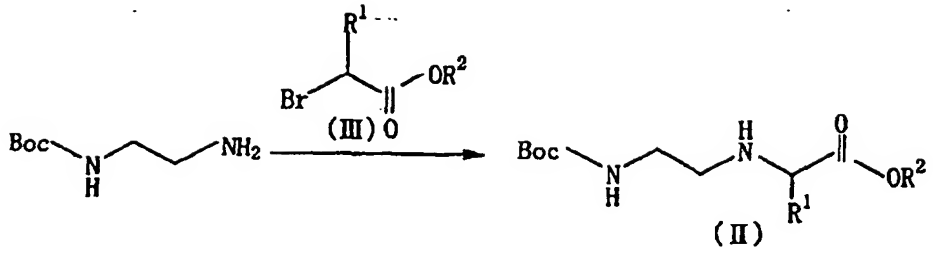
[0052] The first step is the step wherein t-butoxycarboxylic acid anhydride is directly reacted with ethylenediamine using tetrahydrofuran (THF) as solvent without using a reagent to prepare t-butoxycarbonylaminoethylamine.



[0053] t-Butoxycarbonylaminoethylamine can be obtained in a high yield of 98% without producing di-(t-butoxycarbonylamino)ethylene as a byproduct. Since t-butoxycarboxylic acid produced as the byproduct is removed from the reaction system by forming itself into salts insoluble in THF with excessively existing ethylenediamine, purification by partition chromatography is unnecessary.

[0054] Therefore, it is possible to prepare simply t-butoxycarbonylaminoethylamine in a high yield, and further application to a large scale production is easy due to unnecessariness of purification by partition chromatography. Further, since excessively existing ethylenediamine and THF used as the solvent can be reused after distillation, it is possible to attempt a production cost reduced, making use of resource effectively. Further, in the above reaction the reaction condition is preferably under nitrogen atmosphere at room temperature.

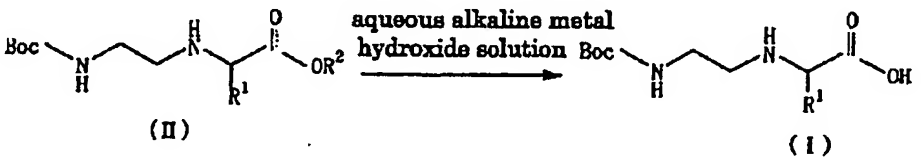
[0055] The subsequent step is the step that the compounds shown by the general formula (II) are prepared by the reaction of t-butoxycarbonylaminoethylamine and the esters shown by the formula (III).



[0056] In the above reaction, though as solvents that can be used are chloroform, THF and the like, use of methylene chloride is preferable. Also, as a catalyst tertiary amine, especially triethylamine, diisopropylethylamine and the like can preferably be used. The reaction condition is preferably under a nitrogen atmosphere at room temperature.

[0057] Although it is determined that the amino acid derivatives shown by the formula (I), which are the final products, become any amino acid derivative in accordance with the type of R^1 in the esters shown by the formula (III), the yield of the above reaction is reduced by steric hindrance depending on the type of R^1. Therefore, R^1 is preferably a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, more preferably a hydrogen atom or a straight chain or branched chain alkyl group with 1-4 carbon atoms, furthermore preferably a hydrogen atom, a methyl or ethyl group. Further, R^2 is preferably a straight chain or branched chain alkyl group with 1-4 carbon atoms, more preferably a methyl, ethyl, n-propyl or isopropyl group, furthermore preferably an ethyl group.

[0058] The subsequent step is the step that the amino acid derivatives shown by the formula (I) are prepared by hydrolysis of the compounds shown by the formula (II).



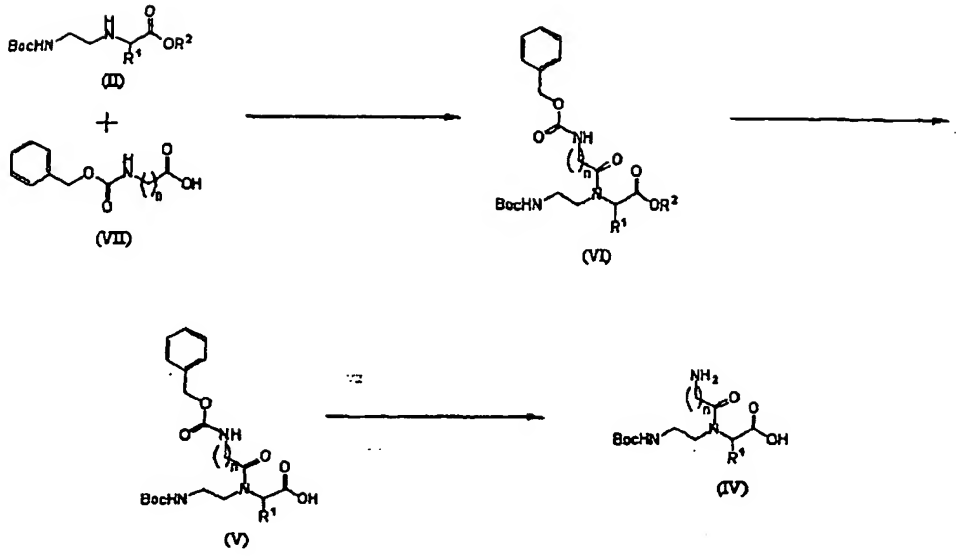
[0059] The hydrolysis is preferably carried out in an aqueous alkaline metal hydroxide solution. As an alkaline metal, lithium, sodium or potassium are preferable, and sodium is most preferable.

[0060] Although the amino acid derivatives shown by the formula (I) are obtained in a form of sodium salts by the above reaction, a sodium ion can easily be removed by cation-exchange chromatography. Further, since a t-butoxycarbonyl group is unstable toward cation-exchange chromatography, it is preferable to remove an alkaline metal ion by cation-exchange chromatography using a pyridinium ion instead of proton as a counter ion in view of preventing decrease of yield.

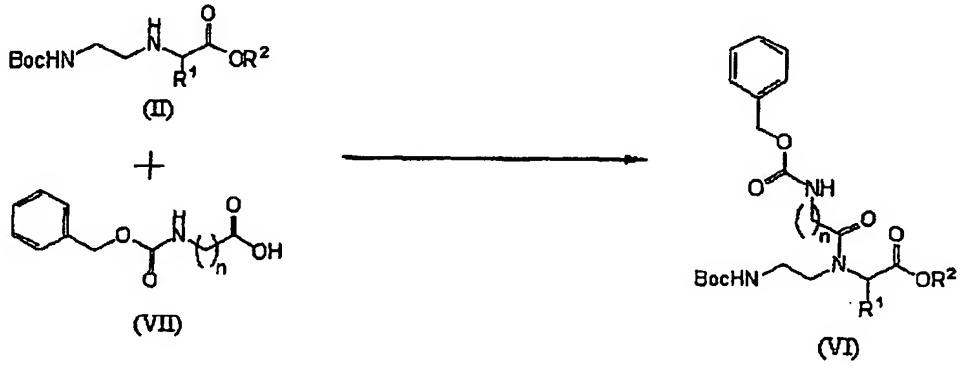
[0061] Since this step is a simple one step reaction called hydrolysis and does not require purification by column chromatography of which application to a mass production is difficult, the amino acid derivatives shown by the formula (I) can be prepared in a high yield, and a further application to an industrial production is easy.

[0062] The process of t-butoxycarbonylethyaminoethylamine and the amino acid derivatives of the invention does not use alkaline conditions. In the meantime, there are many functional molecules except bases constituting a nucleic acid, which are unstable toward an alkaline condition. Therefore, the process of the invention is preferably used in case of preparing amino acid derivatives in aiming their use as base substances for introducing functional molecules.

[0063] The amino acid derivatives shown by the formula (IV) of the invention are prepared by the steps shown below using the compounds shown in the previous general formula (II).

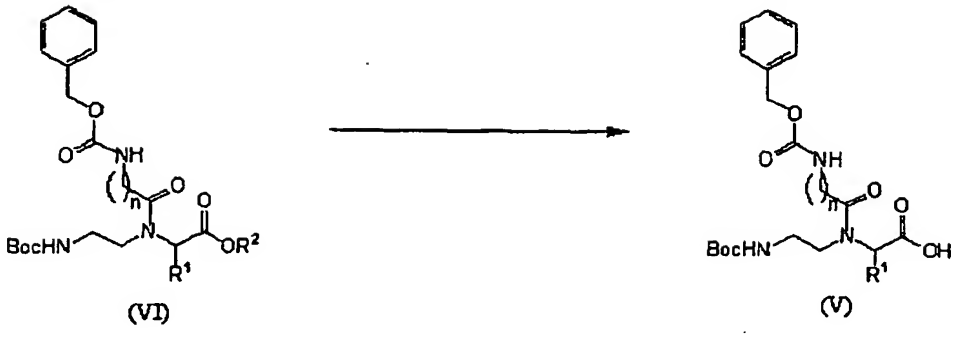


[0064] The first step is , as described below, the step to prepare the benzyloxycarbonyl- -amino acid^{BOC}PNA-OR^2 by the reaction of the compound ^{BOC}PNA-OR^2 shown by the general formula (II) with the benzyloxycarbonyl- -amino acid shown by the general formula (VII) using dimethylformamide (DMF) or the like as the solvent and triethylamine.



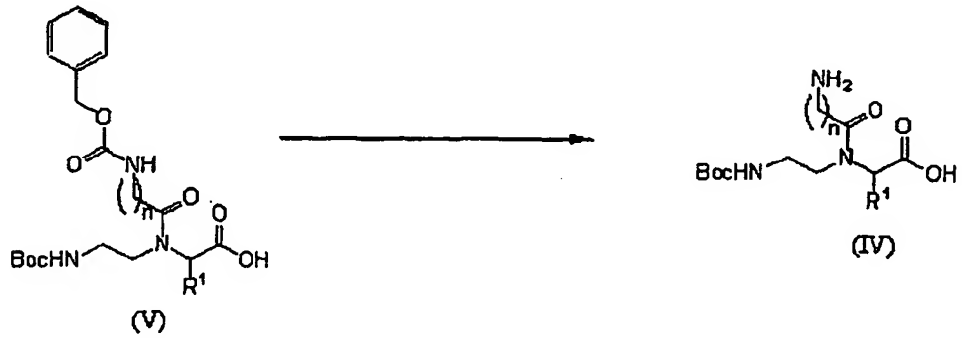
[0065] The solvent DMF, the compounds (II), (VII) and the EDCI derived product can be separable from the aimed substance (VI) by a partition procedure. Since theoretically only the aimed substance (VI) remains in an organic layer, purification by column is unnecessary, though just to be sure purification was done. The aimed substance was obtained quantitatively by this method.

[0066] The subsequent step is the step to prepare the benzyloxycarbonyl- -amino acid-^{BOC}PNA-OH shown by the general formula (V) by hydrolysis of the benzyloxycarbonyl- -amino acid^{BOC}PNA-OR^2 shown by the general formula (VI).



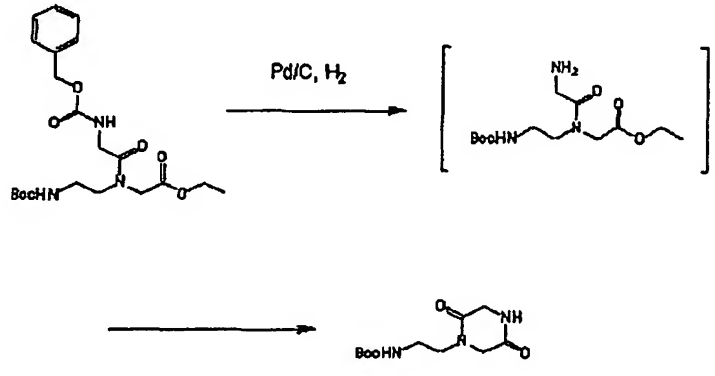
[0067] The hydrolysis is preferably carried out in an aqueous alkaline metal hydroxide solution. As an alkaline metal, lithium, sodium or potassium are preferable, and in particular sodium is preferable. Further, the condition of hydrolysis is under ice-cooling or at room temperature.

[0068] Subsequently, the step to obtain the compound, the final step, shown by the general formula (IV) is carried out.



[0069] This step is preferably carried out in a methanol solution containing palladium carbon as a catalyst.

[0070] As a method to synthesize the compound (IV) from the compound (VI), the method via (V) is the only method. For example, as in the following figures, in case (VI) is first subjected to a catalytic reduction, the cyclic compound is formed via the intermediate.



[0071] Although the amino acid derivative shown by the formula (IV) is obtained in the form of sodium salt by the

above reaction, the sodium ion is easily removed by cation-exchange chromatography. Further, since t-butoxycarbonyl group is unstable toward cation-exchange chromatography, it is preferable that an alkaline metal ion is removed by cation-exchange chromatography using a pyridinium ion instead of proton as a counter ion in view of preventing decrease of yield.

[0072] Since this step is a simple one step reaction called hydrolysis and does not require column chromatography difficult for the application to a mass production, the amino acid derivatives shown by the formula (IV) can be prepared in a high yield, and further the application to a mass production is easy.

[0073] The process of the amino acid derivative of the invention does not use an alkaline condition. In the meantime, there are many functional molecules except bases constituting a nucleic acid, which are unstable toward alkaline conditions. Therefore, the process of the invention is preferably used in case of preparing amino acid derivatives in aiming their use as base substances for introducing functional molecules.

[0074] It is determined that the amino acid derivative shown by the formula (IV), which are the final product, becomes any amino acid derivative in accordance with a type of R^1 and value of n in the ester shown by the formula (VI).

[0075] The yield of the above reaction is reduced by steric hindrance depending on the type of R^1. Therefore, R^1 is preferably a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, more preferably a hydrogen atom or a straight chain or branched chain alkyl group with 1-4 carbon atoms, furthermore preferably a hydrogen atom, a methyl or ethyl group. Further, R^2 is preferably a straight chain or branched chain alkyl group with 1-4 carbon atoms, more preferably a methyl, ethyl, n-propyl or isopropyl group, and furthermore preferably an ethyl group.

[0076] Further, in order to synthesize the compound of the general formula (IV), which is different in n, the corresponding benzyloxycarbonyl- -amino acid can be used. Generally, since the benzyloxycarbonyl- -amino acids of n=1-11 are commercially available, it is easy to obtain them. Names thereof are as follows.

n	Upper column:general name Lower column:rational formula
n = 1	N-Benzyloxycarbonylglycine Z-NH-CH_2-COOH
n = 2	N-Benzyloxycarbonyl-β -alanine Z-NH-$(CH_2)_2$-COOH
n = 3	N-Benzyloxycarbonyl-4-aminobutanoic Acid Z-NH-$(CH_2)_3$-COOH
n = 4	N-Benzyloxycarbonyl-5-aminopentanoic Acid Z-NH-$(CH_2)_4$-COOH
n = 5	N-Benzyloxycarbonyl-6-aminocaproic Acid Z-NH-$(CH_2)_5$-COOH
n = 6	N-Benzyloxycsrbonyl-7-aminoheptanoic Acid Z-NH-$(CH_2)_6$-COOH
n = 7	N-Benzyloxycarbonyl-8-aminooctanoic Acid Z-NH-$(CH_2)_7$-COOH
n = 8	N-Benzyloxycarbonyl-9-aminononanoic Acid Z-NH $(CH_2)_8$-COOM
n = 9	N-Benzyloxycarbonyl-10-aminodecanoic Acid Z-NH-$(CH_2)_9$-COOH
n = 10	N-Benzyloxycarbonyl-11-aminoundecanoic Acid Z-NH-$(CH_2)_{10}$-COOH
n = 11	N-Benzyloxycarbonyl-12-aminododecanoic Acid Z-NH-$(CH_2)_{11}$-COOH

[0077] Among these, Z-glycine, n=1, can in particular preferance, be used.

[0078] Since generally PNA is expected to be a hybrid with DNA, derivatization sterically similar to DNA is desirable. In case the carboxylamino acid is used as a linker, Z-glycine is most preferable considering this point.

Example

[0079] The invention will be illustrated in more detail by way of examples, but the invention is not limited to these examples.

(Example 1) Preparation oft-butoxycarbonylaminoethylamine

[0080] To the THF solution (600ml) of ethylenediamine (90.2g, 1.50mol) was dropped the THF solution (400ml) of t-butoxycarboxylic acid anhydride (32.7g, 0.15mol) in 1 hour, and the mixture was stirred at room temperature for 15 hours. Subsequently, the supernatant is poured and evaporated under reduced pressure at 60°C. The residue was redissolved in THF and dried over $MgSO_4$, followed by filtration. The filtrate was concentrated to give t-butoxycarbonylaminoethylamine 23.7g as a colorless oil. The yield was 98%.

(Example 2) Synthesis of ^{BOC}PNA-OH

[0081] To the THF solution (5ml) of ethyl N-(2-Bocaminoethyl)glycinate (^{BOC}PNA-OEt; 4.52g, 18.4mmol) obtained by reaction of t-butoxycarbonylaminoethylamine with ethyl bromoacetate was

dropped aqueous 2N-NaOH solution (10ml, 20mmol) at 0°C, and then the reaction liquid was stirred at room temperature for 15 hours. This was directly subjected to cation-exchange chromatography (DOWEX 50Wx8, pyridinium form) and eluted with water. The eluate was concentrated under reduced pressure and further dried in vacuum to give N-(2-Bocaminoethyl)glycine (^{BOC}PNA-OH) 3.85g as a white powder. The yield was 96%.

(Example 3) Synthesis of Z-gly-^{BOC}PNA-OEt

[0082] To the dimethylformaamide solution (DMF; 25ml) of benzyloxycarbonylglycine (Z-glycine; 6.75g, 33 mmol) and ethyl N (2-aminoethyl)glycine (4.06g, 17mmol) was

added triethylamine (TEA; 4.78ml, 35 mmol) and the mixture was stirred at 0°C. This was added with 1-ethyl-(3-(3-dimethylaminopropyl)carbodiimide (EDCI; 6.79g, 35mmol) and stirred at 0°C for 2 hours and further at room temperature for 15 hours. The reaction liquid was added with ethyl acetate (EtOAc; 300ml) and sequentially washed with aqueous 5% sodium bicarbonate solution (NaHCO3; 300ml×3), aqueous 5% citric acid solution (300ml×3), aqueous saturated sodium chloride solution (300ml×3). The EtOAc layer was dried over anhydrous magnesium sulfate ($MgSO_4$) and then filtered whereby the filtrate was concentrated. The residue was subjected, to silicagel column chromatography (3% MeOH/dichloromethane) to obtain quantitatively Z-gly-^{BOC}PNA-OEt as a colorless oil. ^{1}H NMR ($CDCl_3$) 7.4-7.2 (m, 5H), 5.77 (brt) and 5.68 (brt) (1H), 5.39 (brs) and 4.97 (brs) (1H), 5.27 (s) and 5.09 (s) (2H), 4.19 (m, 2H), 4.07 (s) and 3.91 (s) (2H), 4.01 (s, 2H), 3.51 (brs) and 3.40 (brs) (2H), 3.34 (brs) and 3.25 (brs) (2H), 1.40 (s, 9H), 1.26 (t, J=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) 169.71 and 169.31 (3), 169.20 and 168.79 (d), 156.11 and 155.85 (d), 136.39 and 136.32 (d), 128.44, 128.27, 127.98, 127.90, 79.80 and 79.37 (d), 66.86 and 66.77 (d), 62.05 and 61.58 (d), 49.43 and 48.73 (d), 48.52 and 48.05 (d), 42.49 and 42.34 (d), 38.48, 28.27, 14.03; FABMS *m/z* 438 [(M+H)$^+$].

(Example 4) Synthesis of Z-gly-^{BOC}PNA-OH

[0083] To the THF solution (20ml) of Z-gly-^{BOC}PNA-OEt (4.0g, 9.2mmol) was

dropped aqueous 1N-NaOH solution (20ml, 20mmol) at 0°C, and the reaction liquid was stirred at 0°C for 1 hour. After the reaction was completed, the reaction liquid was directly allowed to cation-exchange chromatography (DOWEX 50W×8, pyridinium form) and eluted with MeOH. The eluate was concentrated under reduced pressure and further dried in vacuum to obtain Z-gly-^{BOC}PNA-OH (3.09g, 82%) as a colorless oil. ^{1}H NMR (DMSO-*d6*) 7.4-7.2 (m, 5H), 6.84 (brt) and 6.73 (brt) (1H), 5.03 (s) (2H), 4.11 (brs) and 3.94 (brs) (2H), 3.92 (brs) and 3.77 (brs) (2H), 3.33 (brs) and 3.29 (brs) (2H), 3.09 (brs) and 3.02 (brs) (2H),1.37 (s, 9H); ^{13}C NMR (DMSO-*d6*) 171.07 and 170,02 (d), 169.39 and 169.07 (d), 156.42 (brd), 155.70 and 155.61 (d), 137.14, 128.35, 127.77, 127.67, 78.04 and 77.76 (d), 65.38, 48.99, 47.43 and 46.70 (d), 41.92 and 41.52 (d), 38.13 and 37.81 (d), 28.23; FABMS *m/z* 410 [(M+H)+]; HRMS (FAB$^+$) calcd for $C_{19}H_{28}O_7N_3$ [(M+H)$^+$] 410, 1849, observed 410,1926.

(Example 5) Synthesis of Gly-^{BOC}PNA-OH

[0084] To the MeOH solution (20ml) of Z-gly-^{BOC}PNA-OH (4.09g, 10mmol) was

added palladium carbon (5% Pd/C; 100mg), and the catalytic hydrogen reduction was carried out at room temperature. After the reaction was completed, the mixture was filtered through celite. The residue was subjected to sili-

cagel column chromatography (5% MeOH/dichloromethane) to obtain Gly-^{BOC}PNA-OH (2.08g, 75%) as white powder. ^{1}H NMR (DMSO-*d6*) 3.72 (brs) and 3.69 (brs) (2H), 3.58 (brs) and 3.54 (brs) (2H), 3.3-3.2 (m, 2H), 3.06 (brs) and 2.94 (brs) (2H); FABMS *m/z* 276 [(M+H)$^+$].

(Example 5) Synthesis of Dabcyl-Gly-^{BOC}PNA-OH

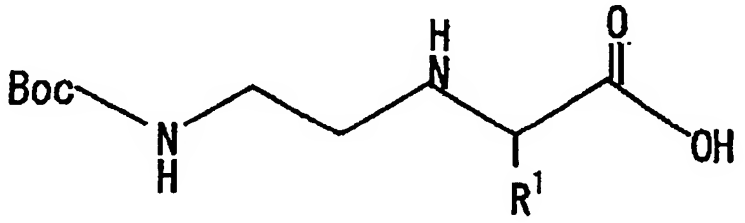
[0085] To the dimethyformamide solution (10ml) of Gly-^{BOC}PNA-OH (100mg, 0.39mmol) was added dabcyl *N*-hydroxysuccinimide ester (145mg, 0.40mmol) and triethylamine (60µl, 0.45mmol) sequentially, and the mixture was stirred at room temperature for 15 hours. After the reaction was completed, the mixture was concentrated under reduced pressure, and the residue was subjected to silicagel column chromatography (0.4% MeOH/dichloromethane) to obtain Dabcyl-Gly-^{BOC}PNA-OH (184mg, 90%) as red brown powder. ^{1}H NMR (DMSO-*d6*) 8.18 (d, J=7Hz, 2H), 7.91 (d, J=7Hz, 2H), 7.88 (d, J=7Hz, 2H), 6.77 (d, J=7Hz, 2H), 5.76 (s) and 5.30 (s) (2H), 4.22 (brs) and 4.05 (brs) (2H), 3.73 (brs) and 3.49 (brs) (2H), 3.47 (brs) and 3.29 (brs) (2H),1.26 (s, 9H); FABMS *m/z* 527 [(M+H)$^+$].

Industrial Applicability

[0086] The process of the invention is able to synthesize t-butoxycarbonylaminoethylamine and Boc type amino acid derivatives whereby it requires no tedious procedure and is also good in yield, and the application to a mass production is easy. Therefore, it can be used for an industrial synthesis of t-butoxycarbonylaminoethylamine and Boc type amino acid derivatives, and utilized for the establishment of a PNA synthesis method using Boc type monomer units, and in the industries of an industrial synthesis of Boc type amino acid derivatives, an industrial synthesis of PNA monomer units using these and so on.

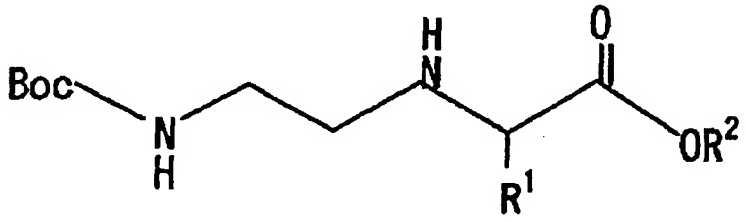
Claims

1. A process of amino acid derivatives shown by the formula I below:



(I)

(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms.), comprising a step to obtain the amino acid derivatives shown by the general formula (I) by hydrolysis of compounds shown by the general formula (II)



(II)

(wherein R^1 has the same meaning, and R^2 means a straight chain or branched chain alkyl group with 1-4 carbon atoms.).

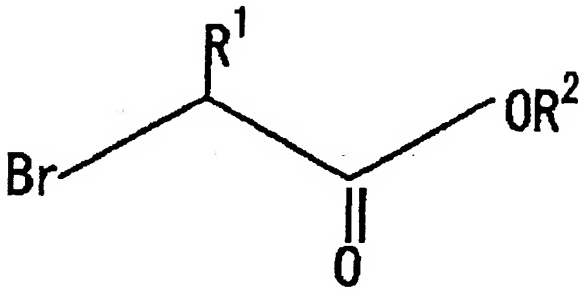
2. The process according to claim 1, **characterized in that** the hydrolysis of the compounds shown by the below

formula (II) is carried out by an aqueous alkaline metal hydroxide solution.

3. The process according to claim 2, **characterized in that** it contains further a step in which the alkaline metal ion is removed by cation-exchange chromatography using pyridinium ion as a counter ion.

4. The process according to claims 2 or 3, **characterized in that** the alkaline metal is lithium, sodium or potassium.

5. The process according to any one of claims 1-4, **characterized in that** the compound shown by the formula (II) is obtained by reaction oft- t-butoxycarbonylaminoethylamine and a compound shown by the formula (III) below:



(III)

(wherein R^1 and R^2 have the same meanings as described above.).

6. The process according to claim 5, **characterized in that** t-butoxycarbonylaminoethylamine is obtained by reaction of ethylenediamine and t-butoxycarboxylic acid anhydride.

7. The process according to claim 5, **characterized in that** the reaction of ethylenediamine and t-butoxycarboxylic acid anhydride is carried out in tetrahydrofuran.

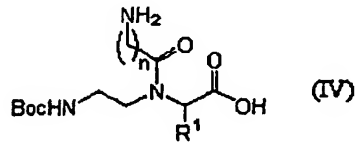
8. The process according to any one of claims 1-7, **characterized in that** in the compounds shown by the general formulas (I), (II) and (III) R^1 is a hydrogen atom and R^2 is an ethyl group.

9. The process according to any one of claims 1-8, **characterized in that** the amino acid derivatives shown the formula (I) are base substances for introducing a base to synthesize a Boc type PNA monomer unit.

10. Use of the process according to any one of claims 1-9 in the preparation of a Boc type PNA monomer unit.

11. The process of t-butoxycarbonylaminoethylamine, **characterized in that** ethylenediamine and t-butoxycarboxylic acid anhydride are reacted in tetrahydrofuran.

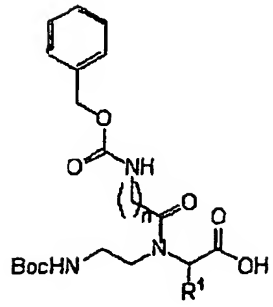
12. Amino acid derivatives shown by the general formula (IV):



(IV)

(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, and n means any one of integers 1-11.).

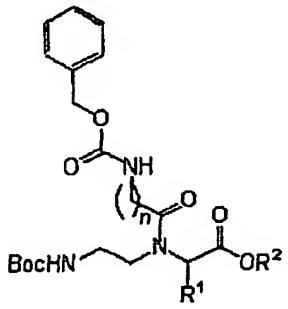
13. Intermediates of the amino acid derivatives shown by the general formula (IV) according to claim 12 which are shown by the general formula (V):



(V)

(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, and n means any one of integers 1-11.).

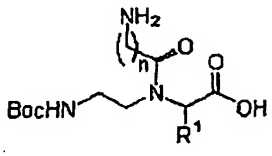
14. Intermediates of the amino acid derivatives shown by the general formula (IV) according to claim 12, which are shown by the general formula (VI):



(VI)

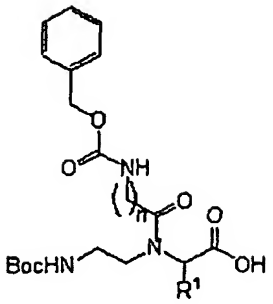
(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, R^2 means a straight chain or branched chain alkyl group with 1-4 carbon atoms, and n means any one of integers 1-11.).

15. The process for the amino acid derivatives shown by the general formula (IV) below:



(IV)

(wherein R^1 represents a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, and n represents an integer of 1-11.) comprising a step to obtain the compounds shown by the general formula (IV) by reduction of the compounds shown by the general formula (V):

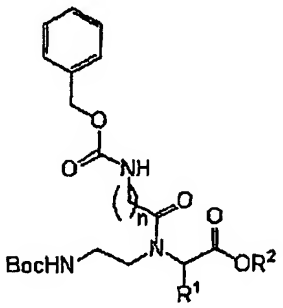


(V)

(wherein R^1 and n have the same meanings as described above.).

16. The process of according to claim 15, **characterized in that** the reduction of the compounds shown by the general formula (V) is carried out in a methanol solution containing palladium carbon as a catalyst.

17. The process of according to claim 16, **characterized in that** it contains a step to obtain the compounds shown by the general formula (V) by hydrolysis of the compounds shown by the general formula (VI):



(VI)

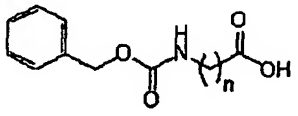
(wherein R^1 and n have the same meanings as described above, and R^2 means a straight chain or branched chain alkyl group with 1-4 carbon atoms.).

18. The process of according to claim 17, **characterized in that** the hydrolysis of the compounds shown by the general formula (VI) is carried out by an aqueous alkaline metal hydroxide solution.

19. The process of according to claim 18, **characterized in that** it contains further a step in which the alkaline metal ion is removed by cation-exchange chromatography using pyridinium ion as a counter ion.

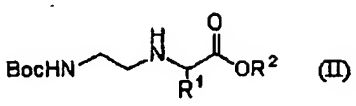
20. The process of according to claims 18 or 19, **characterized in that** the alkaline metal is lithium, sodium or potassium.

21. The process of according to any one of claims 17-20, **characterized in that** the compounds shown by the general formula (VI) are obtained by the reaction of benzyloxycarbonyl- -amino acids shown by the general formula (VII) below:



(VII)

(wherein n has the same meaning as described above.)
and the compounds shown by the general formula (II):



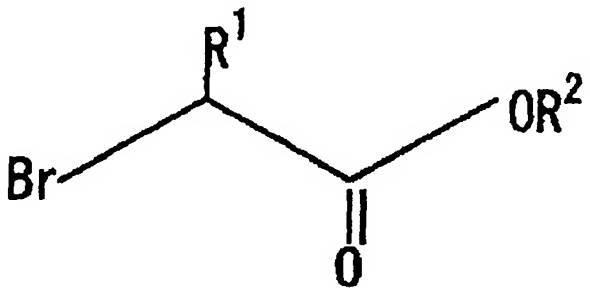
(II)

(wherein R^1 and R^2 have the same meanings as described above.).

22. The process of according to any one of claims 15-21, **characterized in that** in the compounds shown by any one of the general formulas (II) and (IV)-(VII) R^1 is a hydrogen atom, R^2 is an ethyl group, and n is 1.

23. The process of according to any one of claims 15-22, **characterized in that** the amino acid derivatives shown by the formula (IV) are base substances for introducing a base to synthesize a Boc type PNA monomer unit.

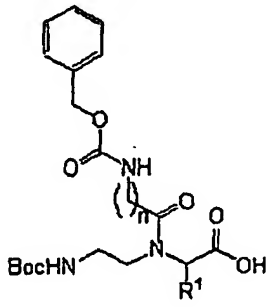
24. The process of according to any one of claims 21-23, **characterized in that** the compounds shown by the general formula (II) are obtained by reaction of t-butoxycarbonylaminoethylamine prepared from ethylenediamine and a compound shown by the general formula (III)



(III)

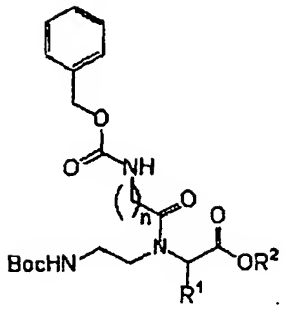
(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl groups with 1-5 carbon atoms, R^2 means a straight chain or branched chain alkyl group with 1-4 carbon atoms, and n means any one of integers 1-11.)

25. The process for the compounds shown by the general formula (V):



(V)

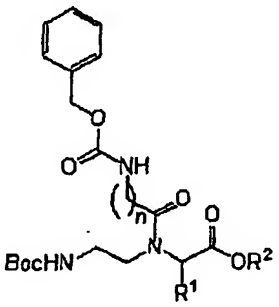
(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, R^2 means a straight chain or branched chain alkyl group with 1-4 carbon atoms, and n means an integer of 1-11.), **characterized in that** it contains a step to obtain the compounds shown by the general formula (V) by hydrolysis of the compounds shown by the general formula (VI):



(VI)

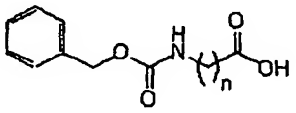
(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, R^2 means a straight chain or branched chain alkyl group with 1-4 carbon atoms, and n means any one of integers 1-11.).

26. The process for the compounds shown by the general formula (VI):



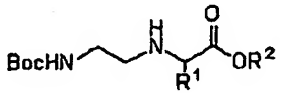
(VI)

(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, R^2 means a straight chain or branched chain alkyl group with 1-4 carbon atoms, and n means any one of integers 1-11.), **characterized in that** it contains a step to obtain the compounds shown by the general formula (VI) by reaction of a benzyloxycarbonyl- -amino acid shown by the general formula (VII):



(VII)

(wherein n means any one of integers 1-11.) and a compound of the general formula (II):



(II)

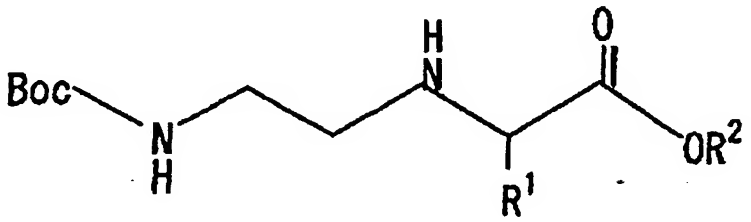
(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl groups with 1-5 carbon atoms, and R^2 means a straight chain or branched chain alkyl groups with 1-4 carbon atoms).

27. Use of the compounds according to claim 12 shown by the general formula (IV) in the preparation of a Boc type

PNA monomer unit.

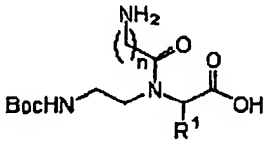
28. Use of the compounds according to claim 13 shown by the general formula (V) in the preparation of a Boc type PNA monomer unit.

29. Use of the compounds according to claim 14 shown by the general formula (VI) in the preparation of a Boc type PNA monomer unit.



(II)

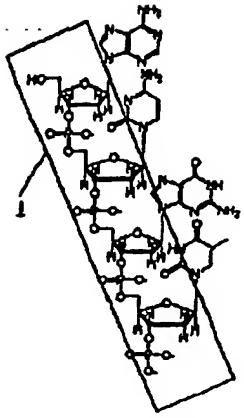
(wherein R^1 has the same meaning as described above, and R^2 means a straight chain or branched chain alkyl group with 1-4 carbon atoms.) as well as amino acid derivatives shown by the general formula (IV):



(IV)

(wherein R^1 means a hydrogen atom or a straight chain or branched chain alkyl group with 1-5 carbon atoms, and n means any one of integers 1-11.), and a process for the amino acid derivative, **characterized in that** it comprises a reduction step of benzyloxycarbonyl- -amino acid derivatives.

Fig. 1 – 1



Natural Nucleic Acid (DNA)

Fig. 1 – 2

Peptide NucleicAcid (PNA)

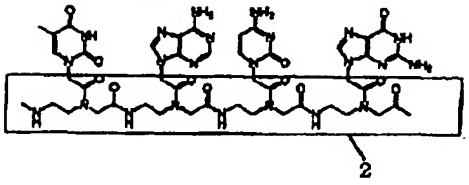
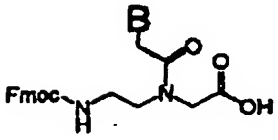
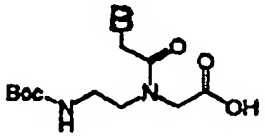


Fig. 2



Fmoc type PNA monomer unit



Boc type PNA monomer unit

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/07696

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The inventions set forth in Claim are divided into the following three groups:

(1) a group of inventions of claims 1-10 relating to processes for the production of amino acid derivatives of the general formula (I),

(2) the invention of claim 11 relating to a process for the production of t-butoxycarbonylaminoethylamine, and

(3) a group of inventions of claims 12-26 relating to amino acid derivatives of the general formula (IV), processes for the production of the derivatives, intermediates for the productions thereof, processes for the production of the intermediates, or use of the amino acid derivatives (IV) or the intermediates.

However, there is no technical relationship among the three groups involving one or more common special technical features, and therefore the three groups of inventions are not considered as relating to a group of inventions so linked as to form a single general inventive concept.

Accordingly, the number of inventions claimed in this international application is 3.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/07696

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] C07C271/20, 269/06, C07K14/00,
B01J39/04, 39/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] C07C271/20, 269/06, C07K14/00,
B01J39/04, 39/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAPLUS (STN), CASREACT (STN), REGISTRY (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99/31121 A2 (PEPTOR LTD.), 24 June, 1999 (24.06.99), & EP 1037904 A2	12-29
A	US 5595741 A (Boehringer Mannheim GmbH), 21 January, 1997 (21.01.97), & JP 4-500361 A & WO 90/15798 A1 & EP 429611 A1	6,7,11
X A	Roeske, Roger W. et al., Selective reduction of the amide carbonyl group in dipeptides by borane, J. Org. Chem., 1976, Vol.41 No.7, pages 1260-1261	1 2-10
A	WO 97/30053 A1 (BIOMEASURE INCORPORATED), 21 August, 1997 (21.08.97), & JP 2001-500838 A & EP 904274 A1	1-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 December, 2001 (10.12.01)	18 December, 2001 (18.12.01)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)